GERSTEL

MAKING LABS WORK

No. 20

solutions worldwide

Water Quality

PFAS Analysis

- Sensory Analysis & Lipid Oxidation
- Dynamic Focusing Air Monitoring
- Sustainability
- Fountain of Youth
- MOSH/MOAH

Spring is in the Air - Software Everywhere

Dear reader,

2024 promises to be a big year for Software with exciting new developments: While MAESTRO continues to be a huge help and support to thousands of users world-wide, operating the widest range of GERSTEL products and solutions, the all new MAESTRO LabFLow software for Client-Server architecture is introduced at analytica 2024 in Munich in April. MAESTRO LabFlow is fully integrated with Agilent® Technologies OpenLab®TM software and will support many routine analysis systems, combining sample preparation and -introduction with GC-MS or LC-MS analysis in a more traceable and networked manner. Also at analytica 2024, new MOSH/MOAH data handling software will be introduced, enabling highly efficient and transparent quantitation, data handling, and customized reporting of MOSH and MOAH results for improved food safety analysis. Last, but not least, the ODI 2 software for Olfactometry combined with the world-wide leading GERSTEL Olfactory Detection Port technology adds exciting new possibilities for efficient quantitation and reporting of olfactory analysis, including panel work. Along with new hardware solutions, we are reporting on GERSTEL's longstanding award winning drive for sustainability, as we have just gone a step further with our recent ISO 14001 certification. In the magazine, several applications are highlighted that bring benefits to laboratories large and small in terms of excellent efficiency and quality of results along with reduced reliance of potentially toxic solvents for extraction and sample preparation. Please never hold back in asking for more information or contact us to discuss how we may help make your laboratory work even more efficient.

Your sincerely,
The GERSTEL team.

CONTENTS

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The new GERSTEL AutoTwister:

Fully automated SBSE / Twister® workflow for solvent free "green" extraction

The GERSTEL AutoTwister revolutionizes the solvent-free extraction process by fully automating the complete workflow from sample extraction to analysis. This fully automated system combines the high degree of automation that SPME offers with the efficiency of the GERSTEL Twister® and provides you with several benefits, such as:

- Excellent sensitivity compared to standard methods (e.g. DIN EN ISO 27108)
- Reduction of manual and repetitive operations
- Minimization of laboratory effort for analysts
- Increase in efficiency
- More sustainable analysis due to reduced consumption of solvents

Experience additional and improved functions, such as integrated barcodes in the Twister rods and comprehensive sample logging which enables traceability as well as improved quality control. The new GERSTEL quickMIX provides cooling and heating capabilities. The specially developed GERSTEL Wash & Dry station improves overall efficiency and sustainability, eliminating potential analyte loss and minimizing water usage. Laboratories can benefit from reduced solvent consumption, reduced environmental impact and most importantly reduced laboratory effort for analysts in the workflow.

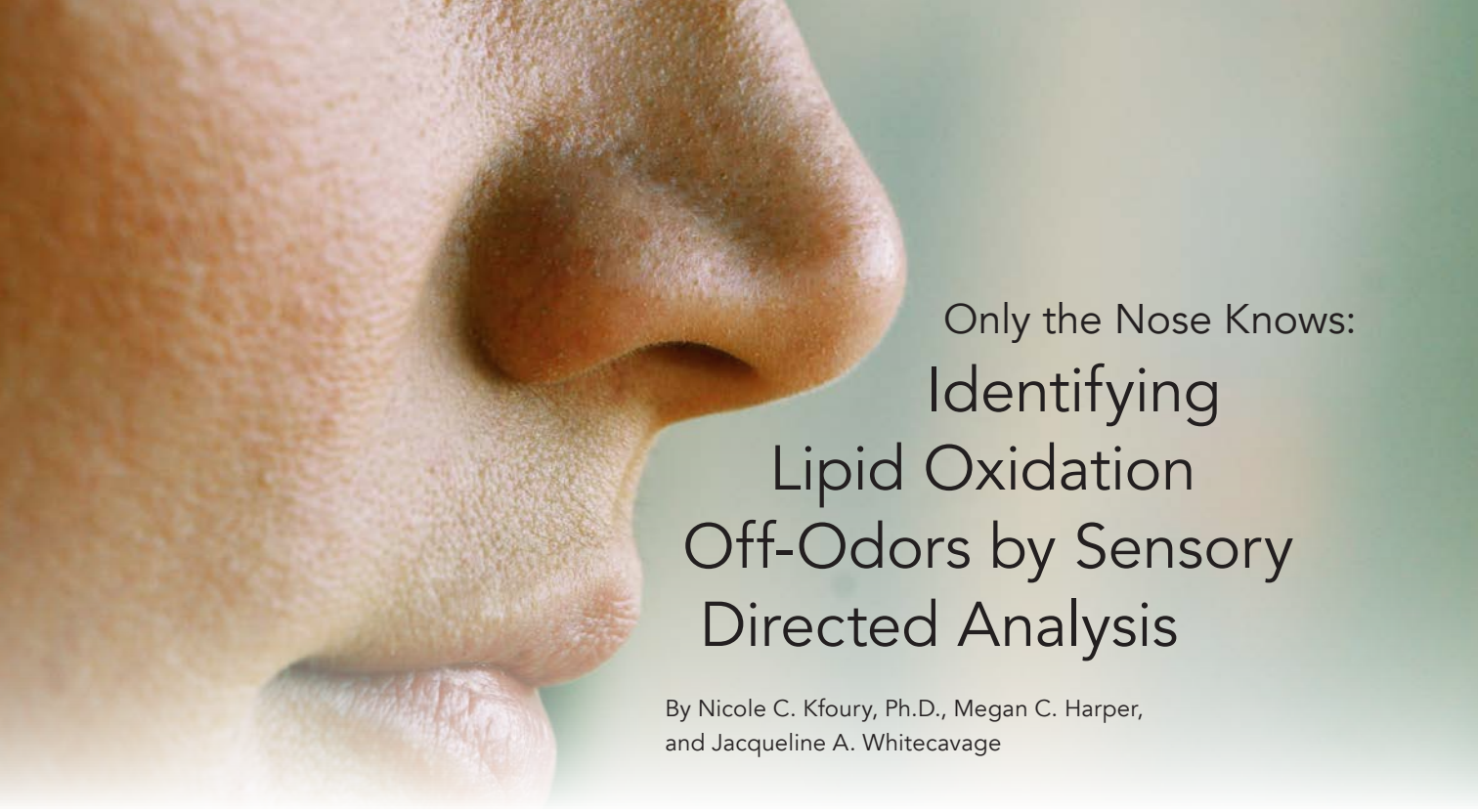
ISDP: Automating Internal Standard and Dry Purge

Maximize the efficiency and performance of your Thermal Desorption analysis!

The all new GERSTEL ISDP (Internal Standard and Dry Purge) accessory for Thermal Desorption (TD) offers fully automated and highly reproducible spiking of 3.5" TD tubes, including GERSTEL 3.5+ tubes, as prescribed e.g. in international standard air monitoring methods. The efficient dry purge function eliminates doubt when analyzing samples with high moisture content, while maintaining high throughput through optimized parallel processing. Two versions are available:

- GERSTEL ISDP for addition of gaseous standard
- GERSTEL ISDP+ for addition of gaseous or liquid standard as selected by the user

Both ISDP versions enable standard spiking and dry purge for 3.5" TD tubes including GERSTEL TD 3.5+ tubes, which contain up to 30% more sorbent than traditional 3.5" tubes. Dry purge is performed in the sampling flow direction to minimize analyte loss. Automated addition of standards extends the scope and efficiency of TD analysis making it more efficient, more accurate and more reliable. The GERSTEL ISDP+ has already proven its worth in air analysis according to U.S. EPA Method TO-17 as well as proficiency tests for DIN ISO 1600-6.



Only the Nose Knows: Identifying Lipid Oxidation Off-Odors by Sensory Directed Analysis

By Nicole C. Kfoury, Ph.D., Megan C. Harper, and Jacqueline A. Whitecavage

Introduction

Lipids are vital to human nutrition, providing energy for biological processes, maintaining brain function, and facilitating the absorption of fat-soluble vitamins [1,2]. Lipid oxidation in food is therefore associated with loss of nutritional value, but as lipids break down, degradation products that are sensory-active even at very low concentrations are also formed, resulting in noticeable rancidity off-odors and reduced customer acceptance[2,3]. Sensory Directed Analysis (SDA) is a process that utilizes gas chromatography in combination with the human nose and mass spectrometry to identify sensory-active compounds. The use of olfactory and MS detection in parallel enables determination of sensory-active regions of the chromatogram and simultaneous mass spectral identification of the associated compounds. As a result, SDA is the ideal technique for solving sensory-related challenges in food products.

In this study, direct thermal extraction (DTE) and dynamic headspace (DHS) were used as automated, solventless means of extracting and concentrating analytes from different sample types. DTE is performed directly in the Thermal Desorption Unit (TDU 2) to determine volatile and semi-volatile organic compounds (VOCs and SVOCs) at trace levels. DHS involves purging the headspace above a solid or liquid sample with inert gas, extracting and then concentrating volatiles on a sorbent-filled trap, resulting in improved recovery and extremely low limits of detection compared with equilibrium headspace. TD Multidesorption Mode can be used to stack analytes from multiple extractions for increased mass on column in areas of interest where no peak signal is initially seen.

Experimental

Instrumentation

GERSTEL MPS LabWorks Platform with Dynamic Headspace (DHS), Thermal Desorption Unit (TDU 2), and Olfactory Detection Port (ODP 4) on Agilent 8890/5977C GC- MSD, GERSTEL Thermal Extractor (TE 2).

Standard Preparation

Standards of hexanal, octanal, nonanal, 2E-octenal, 2E-nonenal, 2E,4E-heptadienal, 2E,4E-nonadienal, and 2E,4E-decadienal were prepared in methanol. One microliter of the standard was spiked onto the glass frit of a glass thermal desorption tube filled with Tenax® TA. Dry nitrogen was passed through the tube for 3 minutes at a flow rate of 50 mL/min to purge the solvent. To confirm the identity of the lipid oxidation off-odor compounds, standards were analyzed using the same instrument conditions.

Sample Preparation

Fresh and aged canola oil, wheat crackers, and cheddar crackers were analyzed to determine the lipid oxidation off-odors. The canola oil was aged at 40 °C for several weeks. Wheat and cheddar crackers were aged at ambient temperature for one year. The very

aged Cheddar cracker sample was aged at 40 °C for one year. A 50 mg sample of canola oil was weighed into a slitted micro-vial, transferred to an empty glass TDU tube, capped with a transport adapter, and placed in a sealed position in a tray on the MPS robotic autosampler for DTE analysis. A 2.0 g sample of each cracker was weighed into individual 20 mL screw-capped vials and placed in the sample tray on the MPS robotic for DHS analysis.

Sample Introduction

Oil samples were extracted by DTE at 90 °C for 15 minutes with a 50 mL/min helium flow. Analytes were trapped in the CIS 4 inlet using a glass bead-filled liner at -120 °C. After the extraction was completed, the trapped analytes were transferred to the GC column in split mode (5:1) by rapidly heating the CIS 4 inlet to 280 °C.

The cracker samples were incubated in the DHS module at 40 °C for 2 minutes and then extracted for 20 minutes at 50 mL/min helium flow for a total purge volume of 1000 mL. Analytes were trapped at 25 °C on a Tenax®TA packed tube. For analysis, the tubes were desorbed in the TDU 2 at 280 °C for 3 minutes with a 50 mL/min helium flow and analytes trapped in the CIS 4 inlet using a glass bead-filled liner at -120 °C. Following tube desorption, the trapped analytes were transferred to the GC column in split mode (5:1) by rapidly heating the inlet to 280 °C.

The GC oven temperature started at 35 °C (2 min), ramping 15 °C/min to 280 °C (2 min). The carrier gas was kept at a constant flow of 1 mL/min and the column used was a 30 m HP-5MS UI, di = 0.25 mm, df = 0.25 µm. The MSD was operated in full scan mode (40-350 amu).

Olfactometry

GC-O analysis was performed with a 2:1 column effluent split between the ODP and MS. The ODP transfer line was heated to 250 °C. The mixing chamber was heated to 150 °C and purged with humidified nitrogen to prevent olfactory fatigue and nasal dehydration.

Results and Discussion

Each sample was initially subjected to direct sensory evaluation to determine the odors of interest associated with lipid oxidation, as shown in Table 1:

Table 1: Sensory characteristics of fresh, aged, and very aged samples

Sample	Fresh	Aged	Very Aged
Canola oil	canola oil, slight green/aldehydic	oxidized, painty, green, aldehydic	n/a
Wheat crackers	cracker, slight green/oily	stale, oily, fatty, oxidized	n/a
Cheddar crackers	cracker, cheddar, slight waxy	stale, oily, fatty, waxy	rancid, fatty, waxy, chemical

The fresh samples exhibited the odors expected from each respective food product, with only very slight oxidation odors. The aged samples had very characteristic lipid oxidation off-odors, including green, painty, oily, fatty, waxy, and rancid. To confirm that DTE and DHS successfully extracted the odors of interest from each sample, a Thermal Extractor (TE 2) was used to release and individually assess the total odor extracted from each oil and and each DHS sorbent trap. The released total extracted odors were assessed by direct olfactory detection a the TE 2 outlet.

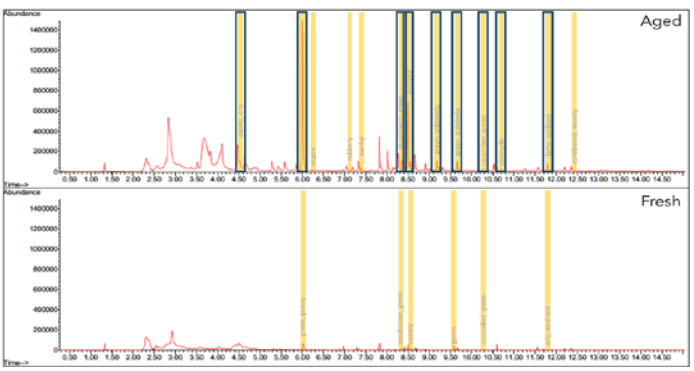


Figure 1: Stacked view of total ion chromatograms of aged (top) and fresh (bottom) canola oil.

Figures 1-2 show the stacked view of aged (top) and fresh (bottom) canola oil and wheat crackers. Figure 3 shows the stacked view of very aged (top), aged (middle), and fresh (bottom) cheddar crackers. The chromatograms in red are overlaid with the olfactory regions in yellow. Odor regions that represent key sensory characteristics determined in the samples are

marked in blue. There are significantly greater peak signals and more odor regions in the very aged and aged samples compared to the fresh samples. The key sensory characteristics detected in each sample, the identified compounds, and the peak areas normalized to the fresh samples are shown in Tables 2-3.

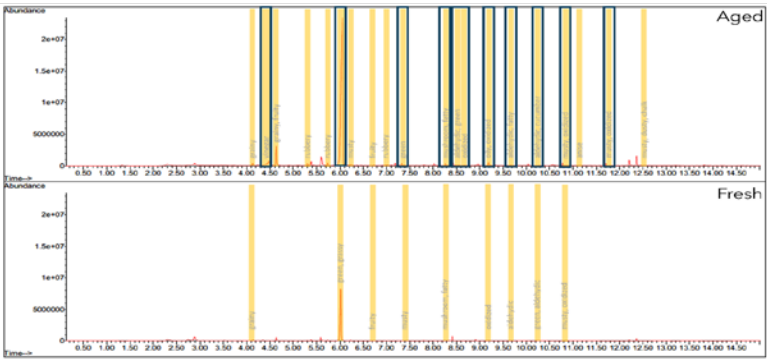


Figure 2: Stacked view of total ion chromatograms of aged (top) and fresh (bottom) wheat crackers.

The aged canola oil was described as oxidized, painty, green, and aldehydic. Oxidized and painty odors detected at the ODP were identified as 1-penten-3-one, 2E,4E-heptadienal, and 2E,4E-decadienal. 2E,4E-heptadienal was detected by the MS in both samples but could only be smelled at the ODP in the aged sample, likely because the concentration in the fresh sample was below the odor threshold. 2E,4E-decadienal, on the other hand, has a very low odor threshold and could be detected at the ODP in both samples, but was below the instrument limit of detection in the fresh sample.
The fresh canola oil had only a slight green and aldehydic aroma; in the aged sample this was much more pronounced. Several green and aldehydic odors were detected at the ODP. Hexanal and nonanal were assessed as green and aldehydic in both samples but were present at much higher levels in the aged sample. 2E-octenal and decanal were also described as green and aldehydic but were only found in the aged samples.
Many of the compounds identified in the wheat crackers were also found in the canola oils. These include hexanal, 1-octen-3-ol, 2E,4E-heptadienal, and 2E-octenal. Some additional compounds were identified, including acetic acid, heptanal, and octanal, as can be seen in Figure 2.The former two were identified in both the fresh and aged samples at the MS but were only detected at the ODP in the aged samples, again suggesting that they are present at a concentration below their odor threshold in the fresh samples and thus not contributing to any off-odor, as can be seen in Table 3.
In three regions, green and oxidized odors were detected at the ODP but not by the MS. The compounds in the two regions around 10.83 and 11.82 minutes were identified by increasing mass on column through the TD Multidesorption Mode, as can be seen in Figure 4. 2E,4E-decadienal was previously identified in the canola oil, but 2E,4E-nonadienal was newly identified in the wheat crackers. The first region, at 10.28 minutes, has the same retention time

Table 2: Key sensory characteristics, identified compounds, and relative peak areas for fresh and aged canola oil.

RT	Odor Characteristic		Compound	Peak Area	
	Fresh	Aged		Fresh	Aged
4.58		painty, oily	1-Penten-3-one	n.d.	1.0
6.01	green, grassy	green, grassy	Hexanal	1.0	19.2
8.26	mushroom, green	mushroom, green	1-Octen-3-ol	n.d.	1.0
8.48		oxidized	2E,4E Heptadienal	1.0	10.8
9.18		green, aldehydic	2E-Octenal	n.d.	1.0
9.65	green	green, aldehydic	Nonanal	1.0	4.8
10.2	cucumber, green	cucumber, green	n.d.		
10.7		aldehydic	Decanal	n.d.	1.0
11.8	oily, oxidized	fatty, oxidized	2E,4E-Decadienal	n.d.	1.0

Note: n.d. = not detected

Table 3: Key sensory characteristics, identified compounds, and relative peak areas for fresh and aged wheat crackers.

RT	Odor Characteristic		Compound	Peak Area	
	Fresh	Aged		Fresh	Aged
4.45		vinegar	Acetic acid	1.0	6.6
6.04	green, grassy	green, grassy	Hexanal	1.0	5.5
7.35		green	Heptanal	1.0	7.6
8.26	mushroom, fatty	mushroom, fatty	1-Octen-3-ol	1.0	8.3
8.58		aldehydic, green	Octanal	n.d.	1.0
8.69		oxidized	2E,4E-Heptadienal	n.d.	1.0
9.22	oxidized	oily, oxidized	2E-Octenal	1.0	4.7
9.68	aldehydic	aldehydic, fatty	Nonanal	n.d.	1.0
10.28	green, aldehydic	aldehydic, cucumber	n.d.		
10.83	musty, oxidized	musty, oxidized	n.d.		
11.82		musty, oxidized	n.d.		

Note: n.d. = not detected

and odor descriptor as the unidentified compound in the canola oil, which could be identified in the very aged cheddar crackers as 2E-nonenal. In addition to the aldehydes detected in the oils and wheat crackers, fatty acids and methyl ketones were found in the cheddar crackers. The fatty acids are found at relatively low levels in the fresh and aged samples, likely due to their natural presence in cheddar [4].

However, the fatty acid levels were drastically increased in the very aged samples, potentially due to exposure to elevated temperatures for a prolonged time. These fatty acids cause the waxy, fatty, and rancid aromas in the very aged crackers. Interestingly, 2E,4E-nonadienal and 2E-4E-decadienal levels increase in the aged compared to the fresh crackers but are no longer present in the very aged sample. It is likely that these compounds are breaking down and forming methyl ketones at the elevated temperature, to which the very aged sample was exposed[1]. The methyl ketones also contribute to the fatty and rancid odors in the very aged sample. For more detailed information, please see the associated GERSTEL AppNote[5].

Conclusion

Sensory Directed Analysis (SDA) can help identify key sensory-active off-odor compounds formed by lipid oxidation. The data shows distinct differences in chromatography and sensory perception between fresh, aged, and very aged samples[5]. Notably, the presence or absence of a compound in the MS chromatogram does not prove or disprove its sensory impact on the sample. Many compounds were detected by the MS but produced no detectable odor at the ODP. In contrast, several compounds were smelled at the ODP, but no peak signal was seen. Invaluable information is missed in an MS-only approach. The SDA approach can readily be used for a wide variety of applications to identify sensory-active compounds. SDA is a critical tool when creating high-quality food products and working to maintain brand loyalty and customer satisfaction.

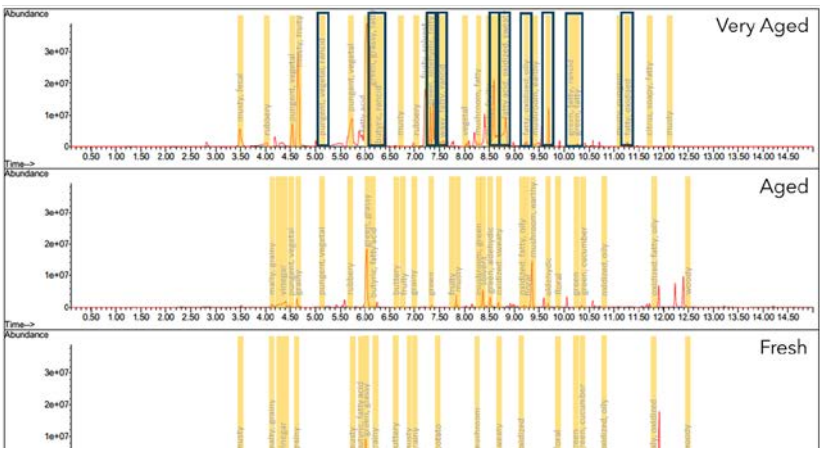


Figure 3: Stacked view of total ion chromatograms of very aged (top), aged (middle), and fresh (bottom) cheddar crackers.

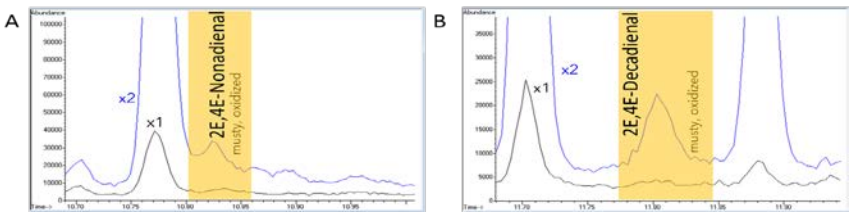


Figure 4: TD Multi-Desorption mode for identification of 2E,4E-nonadienal (A) and 2E,4E-Decadienal (B).

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[1] S. Grebenteuch, C. Kanzler, S. Klaussnitzer, L. W. Kroh, and S. Rohn. The formation of methyl ketones during lipid oxidation at elevated temperatures. Molecules 26 (2021).

[2] S. Böttcher, U. Steinhäuser, S. Drusch. Off-flavor masking of secondary lipid oxidation products by pea dextrin. Food Chemistry 169 (2015) pp. 492-498.

[3] P. Goméz-Cortés, G. L. Sacks, J. T. Brenna. Quantitative analysis of volatiles in edible oils following accelerated oxidation using broad spectrum isotope standards. Food Chemistry 174 (2015) pp. 310-318.

[4] Hayaloglu A. A., Karabulut, I. Characterization and comparison of free fatty acid profiles of eleven varieties of Turkish cheeses. Int J Food Prop 16 (2013) pp. 1407-1416.

[5] Nicole C. Kfoury, Megan C. Harper, and Jackie A. Whitecavage. GERSTEL AppNote: Identification of Off-Odor Compounds Associated with Lipid Oxidation in Food Products Using Sensory Directed Analysis.

SDA Workshops in the US and Germany

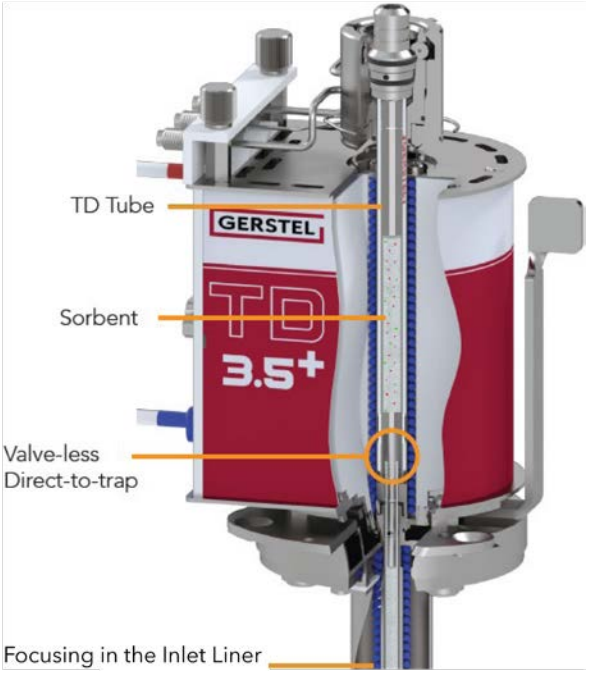
GERSTEL's 3-day SDA Workshop is designed for analytical chemists and sensory scientists. The workshop integrates sensory and instrumental analysis for investigating sensory-active compounds to solve critical challenges in a wide variety of products and sample types. It includes lectures, hands-on demonstrations, and interactive discussions with our Analytical Services Group. Interested? Please contact: workshop@gerstel.de

Air Monitoring and Material Emissions

Dynamic Focusing: A Novel Cryogen-Free Technique for Determining VVOCs, VOCs, and SVOCs

Dynamic Focusing for Thermal Desorption is a novel technique in the field of air monitoring and material emissions analysis that eliminates the need for cryogenic cooling while delivering exceptional results. Dynamic Focusing enables analytical laboratories to determine very volatile, volatile, and semi-volatile organic compounds (VVOCs, VOCs, and SVOCs) with high accuracy and precision, low limits of detection, and high efficiency.

The graphics on the next page illustrate the dynamic focusing process.



VVOC, VOC, and SVOC analysis by TD

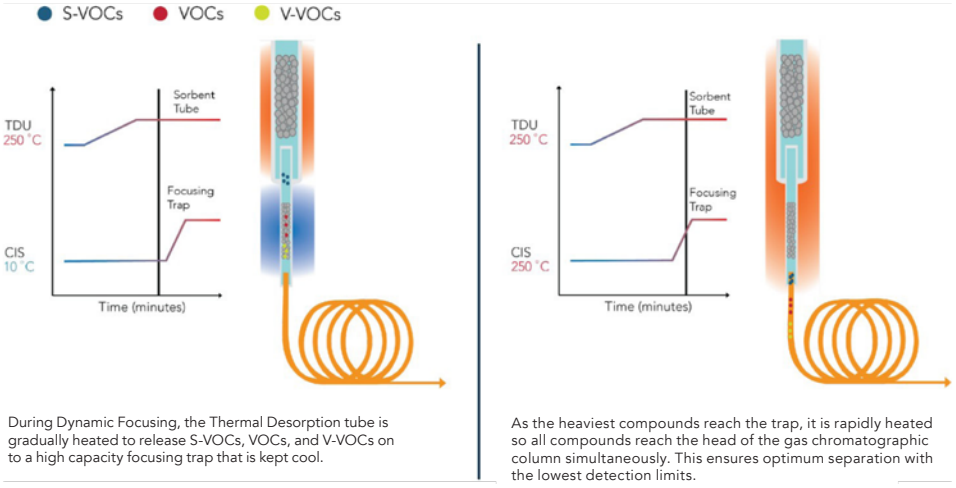
Dynamic Focusing is a novel cryogen- free technique, integral to the GERSTEL TD Core system, developed for use in thermal desorption based analysis of air. Dynamic Focusing is used to focus and trap VVOCs, VOCs, and SVOCs before releasing them to the gas chromatography (GC) column in a sharp band for analysis. The Dynamic Focusing technique has successfully undergone rigorous testing, including compliance with methods like U.S. EPA TO-17 and ISO 16000-6, and it has excelled in inter-laboratory proficiency tests.

Understanding Dynamic Focusing:

Dynamic Focusing diverges from traditional thermal desorption methods in key ways: A single, relatively weak sorbent is packed into the trap liner, and the trap temperature is set to +10 °C. Instead of trapping and desorbing in two separate stages, these are overlapped:

Medium to high boilers are trapped by the sorbent and desorbed upon heating, while very volatile organic compounds (VVOCs) are slowed down just enough to be focused into a sharp band, resulting in remarkably sharp peaks.

Precise timing of temperature and flow provides optimum separation

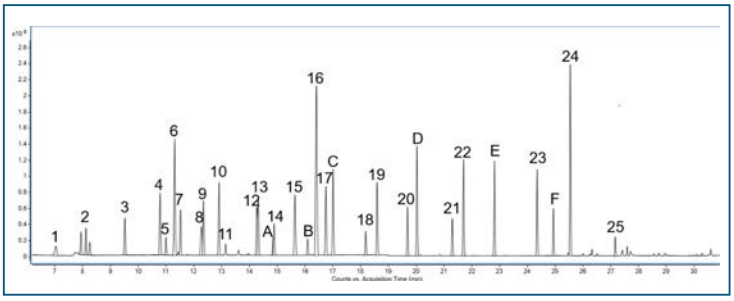


During Dynamic Focusing, the Thermal Desorption tube is gradually heated to release S-VOCs, VOCs, and V-VOCs on to a high capacity focusing trap that is kept cool.

As the heaviest compounds reach the trap, it is rapidly heated so all compounds reach the head of the gas chromatographic column simultaneously. This ensures optimum separation with the lowest detection limits.

Benefits of Dynamic Focusing:

- Streamlined system Design: The TD tube and trap are directly connected (liner-in-liner) and the GC column is directly inserted into the trap, minimizing void volume, and eliminating the need for valves or transfer lines. This design reduces analyte contact with surfaces that can impact recovery and cause carry-over.
- PFAS-Free Flow Path: Absence of PTFE (Teflon™) material in the analyte flow path eliminates the potential for introduction of PFAS-related compounds as well as the loss of these compounds by sorption into PTFE, which are crucial aspects of today's environmental analysis methods.
- Cryogen-Free Cooling: Peltier elements cool the trap to +10 °C, focusing compounds as volatile as propylene (C3) and covering the full range of analytes for standard air analysis methods
- User-Friendly-Low Maintenance: Easy to use, maintain, and troubleshoot, resulting in improved system uptime
- Wide Range of Applications: Dynamic Focusing is suitable for a range of Standard Methods, including US EPA TO-17, ISO 16000-6, and ASTM D6196



Chromatogram of a TO-17 gas standard (numerically labeled) and spiked gas internal standards (alphabetically labeled). Based on Dynamic Focusing and full scan MS detection.

Cryogen-Free Dynamic Focusing for Thermal Desorption, in a system without valves or transfer lines, introduces a new level of performance and simplicity to air monitoring. This innovative technique offers researchers and environmental scientists a powerful tool to determine very volatile to semi-volatile organic compounds with high accuracy and precision combined with high efficiency. By eliminating both the need for cryogenic cooling and valves & transfer lines in the sample pathway. Dynamic Focusing streamlines the entire workflow for targeted VVOC, VOC, and SVOC determination, reliably delivering high quality data while reducing maintenance cost and system downtime.

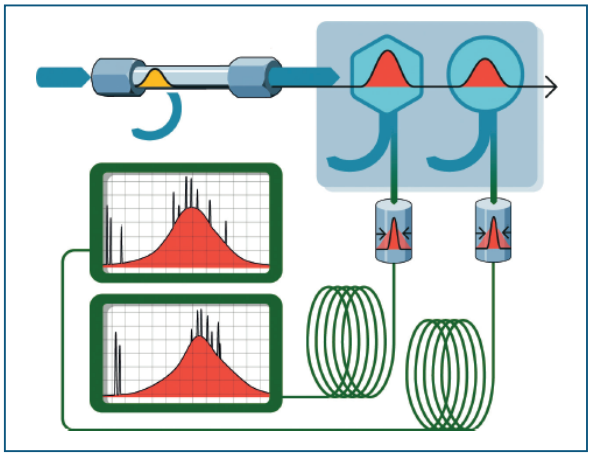
Quality Assurance and Consumer Protection

MOSH/MOAH analysis - efficiently automated

Instrumental analysis is a critical pillar of consumer protection, especially for food safety. To ensure a reasonable cost benefit balance, high laboratory productivity and good quality of results, leading contract laboratories increasingly strive to automate their processes. An example is the determination of mineral oil residues in food, food packaging, and cosmetics.

By Oliver Lerch, Ph.D. and Isabelle Heker

Industrial production, processing and transportation invariably put food at risk of contamination with mineral oil hydrocarbons, which are subdivided into two chemically distinct fractions: MOSH (Mineral Oil Saturated Hydrocarbons) and MOAH (Mineral Oil Aromatic Hydrocarbons). According to the European Food Safety Authority (EFSA), the MOAH fraction contains highly potent carcinogenic compounds, and both fractions accumulate in human tissue. From a toxicological standpoint and in the best interest of the consumer, food, and food contact materials, which mainly means food packaging, should be monitored for the presence of MOSH and MOAH[1]. The same applies to cosmetics, which are of course in direct contact with our skin.



Principle of MOSH/MOAH determination by LC-GC-FID. Normal phase LC separation of MOSH and MOAH and subsequent parallel GC-FID analysis.

GERSTEL MPS robotic-HPLC-GC-FID solution for automated epoxidation and MOSH/MOAH determination.

Sizing up the application details

In MOSH/MOAH analysis, the main points are to extract mineral oil hydrocarbons from the sample and subsequently to determine them using a suitable analysis method. MOSH/MOAH analysis involves several extract cleanup steps to separate, concentrate, and transfer the MOSH and MOAH fractions from the sample matrix into a solution that can be analyzed in a sensitive and robust manner, yielding accurate results. Interfering matrix compounds such as triglycerides are removed automatically on the HPLC column during separation of the fractions.

The hydrocarbons contained in the MOSH and MOAH fractions are determined separately, but simultaneously, in a dual channel gas chromatography system using flame ionization detection (GC-FID).

Depending on the sample type and matrix, additional sample preparation steps may be required to eliminate naturally occurring hydrocarbons from the extract. Some samples must be epoxidized prior to the LC-based fractionation to remove unsaturated polyenes that would otherwise interfere with the MOAH determination. Depending on the matrix, the MOSH fraction must be subjected to a further cleanup step using an aluminum oxide (AlOx) column to remove n-alkanes of plant origin.

To sum it up, determining MOSH and MOAH residues in food or cosmetics is a highly labor intensive task when performing the sample preparation manually. Contract laboratories and routine laboratories that are measured not only by the quality of their results, but also by their productivity and by how fast results are delivered, are well advised to automate the process.

Focus on the customer benefit

With the aim of maximizing efficiency, productivity, accuracy, and precision of the MOSH/MOAH analysis, GERSTEL has developed an integrated system that automates sample preparation, including extract cleanup, and LC-GC-FID determination of both fractions.

The hardware consists of an Agilent 1260 Infinity II HPLC system and a dual channel GC-FID system (Agilent 8890 GC) coupled by an LC-GC interface (GERSTEL) and combined with a MultiPurpose Sampler (GERSTEL MPS robotic). The system is highly modular and easily adapted to changes in requirements. Systems based on the MPS Single Head autosampler fulfill all requirements for efficient automated determination of the MOSH and MOAH fractions in prepared extracts optionally including AlOx cleanup. Dual Head systems additionally enable cleanup and sample preparation steps such as saponification and epoxidation using performic acid or *m*-Chloroperoxybenzoic acid (mCPBA), including high energy agitation, centrifugation, evaporative concentration, cool storage of reagents and samples, wash stations for syringes, and more. Both Single- and Dual Head MPS systems enable maximum efficiency in performing all sample preparation and introduction steps. Optionally, a dedicated module for AlOx cleanup of the MOSH fraction can be added.

LC-GC coupling

When developing the LC-GC coupling, we focused on ruggedness and ease of maintenance of the entire system to maximize system up-time, sample throughput, and laboratory productivity. Our concept includes a special GC column mounting system that enables unfettered access to columns and connectors for fast and easy replacement and the column connector technology used ensures long-lasting gas

tight connections even after multiple instances of disconnecting and reconnecting columns. The user has full control of all system parameters and pre-columns are easily rinsed or replaced as needed. The solvent vapor venting system (GERSTEL Early Vapor Exit, EVE) uses a novel approach: Valves are unheated for simplicity and for easy replacement of capillary connectors to the EVE. An integrated purge system removes residual solvent from the valve and connected tubing for long-lasting rugged operation.

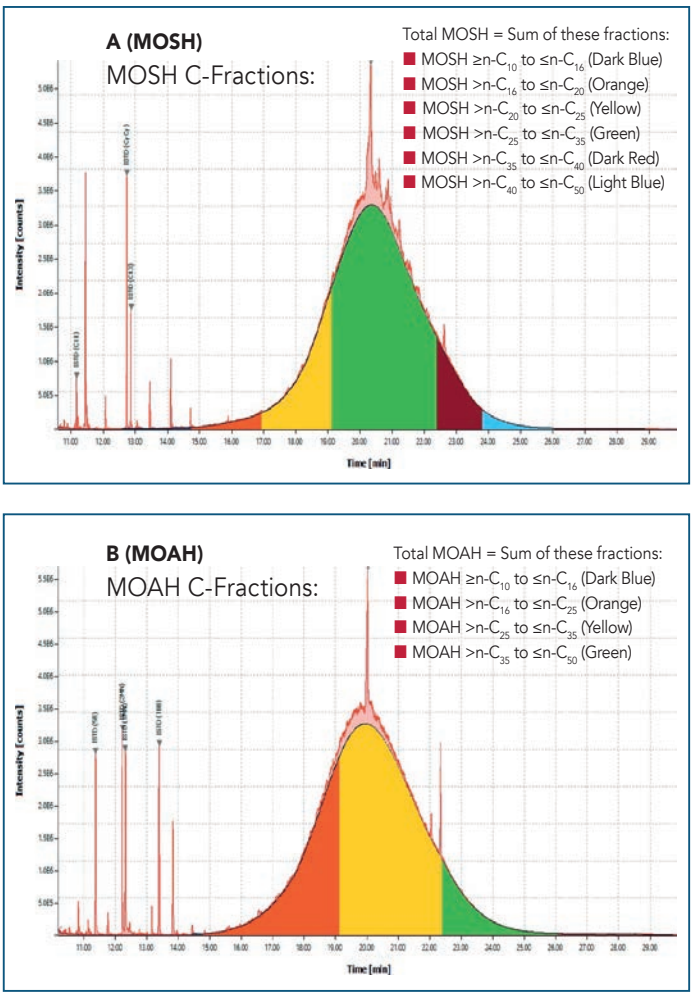


Figure 2: Unresolved complex mixtures (UCM) of MOSH (A) and MOAH (B) fractions extracted from an edible oil sample and integrated using the GERSTEL MOSH/MOAH Data Analysis software. The colored peak (signal) areas represent individual c-fractions. The total amounts are calculated as the sum of all c-fractions between n-C10 and n-C50. The values are included in the final report. System control of the GERSTEL MPS robotic-HPLC-GC-FID solution for MOSH/MOAH analysis is performed through the MAESTRO software; data handling is simplified using a dedicated software.

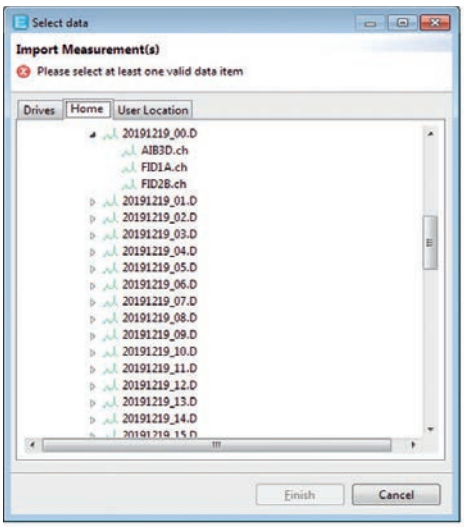
Analysis Details

A quick summary of the analysis: Sample cleanup and fractionation is performed using normal phase liquid chromatography (NP-LC), based on polarity differences. The MOSH fraction elutes before the MOAH fraction using a solvent gradient consisting of *n*-hexane (C_6H_{14}) and dichloromethane (CH_2Cl_2). Matrix residues are backflushed from the column using a flow of 100 % dichloromethane before the column is reconditioned and prepared for the following sample with a forward flow of *n*-hexane. A UV detector set to 230 nm is used to verify separation and transfer to the GC of the two fractions. The UV detector cannot be used for quantification of the fractions since many of the compounds contained, and the internal standards [3], are insufficiently UV-active and do not deliver equimolar responses. Following HPLC separation, the large volume fractions (450 µL each) are transferred directly to the dual channel GC system with two GC columns in the oven and two FID detectors for simultaneous determination of the MOSH and MOAH fractions. Initially, the fractions are focused and prepared for GC analysis by evaporating the solvent and venting the fumes through the specially designed EVE system. Following GC separation, analytes are detected by FID, a detector type that delivers well known mass responses for the range of analytes contained in the MOSH and MOAH fractions. Quantitation is performed as the sum of all C10-C50 compounds with individually added sections in accordance with the JRC Guidance [3]. The internal standard mixture used for MOSH/MOAH analysis includes nine different compounds. These are used for quantification and as markers and controls for both the HPLC fractionation and the GC separation performance. A separate „retention time standard" with 10 compounds from C10 to C50 is analyzed regularly to determine the prescribed size ranges.

Benefits of Automation

The description above illustrates the complexity of the MOSH/MOAH sample preparation. Automation reduces the risk of errors while improving efficiency, reliability, and reproducibility on a 24/7 basis. Further, the ease of operation and the process miniaturization result in improved sustainability while freeing up analyst time to focus on more pressing tasks such as planning, data evaluation and reporting. All parts of the MOSH/MOAH analysis system are under unified control of the MAESTRO software integrated with

the Agilent OpenLab CDS. Operation is user friendly and intuitive. Data handling has been simplified by a dedicated software package for automated MOSH/MOAH data handling. "Saddle peaks" resulting from naturally occurring compounds are clearly visible on top of the MOSH and MOAH humps consisting of thousands of mineral oil related compounds. The size of each hump is decisive, the peaks on top do not count, they are automatically disregarded or subtracted by the software. The humps are automatically integrated with a blank chromatogram used as the lower baseline. The results are reported for each size range as well as for the whole C10 - C50 range. Manual correction of integration parameters is possible at every step, if required. The software enables the user to change integration parameters manually as needed after inspecting the results followed by efficient automated batch reintegration of an entire series of analysis results.



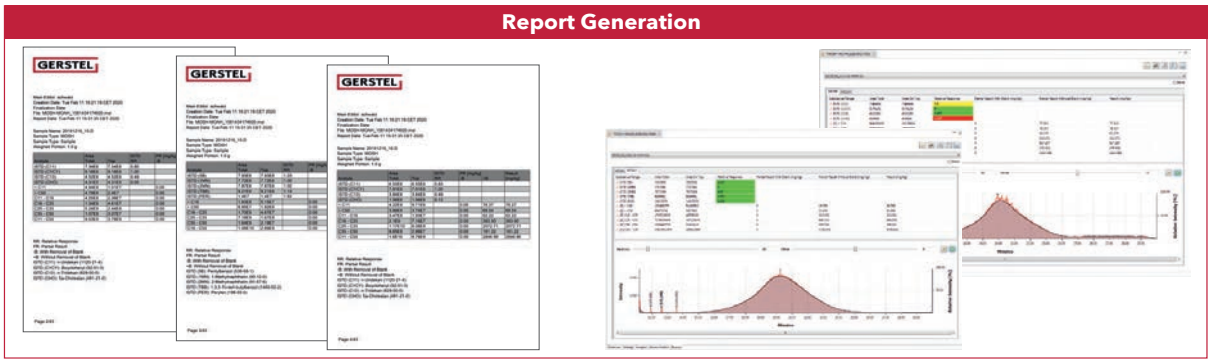


Figure 3: Report Generation: Results are presented in one of several standard report formats that are easily customized to meet individual needs of the laboratory. Data can be exported in a variety of formats for further processing.

Final Words

The fully automated GERSTEL MOSH/MOAH solution meets the requirements of international standard methods. The fractionation is rugged and reliable, good recovery of the n-alkanes up to C50 as percentage of the n-C20 is achieved, and no significant carry over is seen. Further, the system is reliable for routine operation, meeting all requirements for occupational safety, data integrity, intuitive operation, and ease of maintenance. Obtained analysis results correspond well with reference analyses performed by independent laboratories, which have successfully taken part in round robin tests.

References:

[1] European Food Safety Authority (EFSA), Scientific Opinion on Mineral Oil Hydrocarbons in Food, EFSA Journal 2012;10(6):2704, http://bit.ly/2TnvTWb

[2] DIN EN 16995 Foodstuffs - Vegetable oils and foodstuff on basis of vegetable oils - Determination of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) with on-line HPLC-GC-FID analysis EN 16995:2017-08

[3] JRC Technical Reports: Guidance on sampling, analysis and data reporting for the monitoring of mineral oil hydrocarbons in food and food contact materials (in the frame of Commission Recommendation EU 2017/84), http://dx.doi.org/10.2760/208879

Automated MOSH/MOAH Sample Prep

Three sample preparation steps are generally used for MOSH/MOAH analysis:

1) Aluminum oxide cleanup
2) Epoxidation
3) Saponification

Each of these methods handles a specific challenge in MOSH/MOAH analysis and can be used independently. It is possible to use only epoxidation or only aluminum oxide cleanup on one sample type, but it is also possible to use all of them consecutively on the same sample.

Cleanup with activated Aluminum oxide (AlOx):
Using activated aluminum oxide allows the removal of n-alkanes of plant origin from the MOSH fraction that might otherwise interfere with integration of the hump when evaluating the data. The n-alkanes are retained on the AlOx column and separated from the target compounds. In traditional manual sample preparation, single-use glass columns are used. The aluminum oxide has to be activated at 400 °C for 40 h and is discarded after every sample. Unfortunately, MOAH compounds are retained on the column as well and the AlOx columnn must be discarded after each manual cleanup step. As a consequence, when using manual sample preparation, two complete analyses are required for each sample: One for MOSH with AlOx preparation and one for MOAH without AlOx.

The GERSTEL solution is online AlOx cleanup after separation of the MOSH and MOAH fractions, preventing loss of the MOAH analytes and eliminating the need for a separate MOAH determination: Aluminum oxide is packed in a column, similar to an LC column, that lasts a few hundred samples (depending on the matrix) and is connected to the LC system via a second LC pump. While the MOSH fraction is passed through the AlOx column, the MOAH fraction is "parked" in an injection loop. Following cleanup, both fractions can be injected into the GC simultaneously, producing MOSH and MOAH results in a single instrument run. Additionally, this procedure saves immense amounts of solvent and single-use raw materials compared to the manual procedure.

Epoxidation: Epoxidation is used to derivatize olefins to epoxides. Olefins are mainly of plant origin and may interfere with the MOAH analysis. Epoxidation increases their polarity and thereby their retention on the HPLC column causing them to elute after the MOAH fraction. Epoxidation is performed by the MPS using a GERSTEL quickMIX, a centrifuge, and a decapper. The decapper is needed to eliminate siloxane contamination of the sample, resulting from multiple septum penetrations during sample preparation. The process is performed on-line in the system prior to sample injection. Epoxidation can be combined with saponification on a stand-alone MPS WorkStation used for off-line sample preparation.

Saponification: Samples with high fat content may need to go through saponification to remove excess fat before introduction to the analysis system and prevent triglyceride breakthrough in the LC column. The reaction is fully automated using the MPS with a GERSTEL quickMIX, GERSTEL mVAP multi-position evaporation station, Agitator, and Centrifuge to name the main options used. The system is modular and easily changed or upgraded with additional modules as needed when requirements change.

Saponification is performed in batches of up to six samples for maximum efficiency on a stand-alone MPS WorkStation and can be combined with epoxidation for a total throughput of 36 samples per day without limiting the sample capacity of the MOSH/MOAH analysis system.

Water analysis

Monitoring PFAS

Per- and polyfluorinated alkyl substances, or PFAS in short, are ever present and persistent in water. Some members of this huge substance class fall under the Drinking Water Directive. The analysis only requires a single milliliter of water and very little solvent - when online SPE is used.

By Thomas Brandsch, Ph.D.

Per- and polyfluorinated alkyl substances (PFAS) form a family of highly fluorinated organic chemicals, in part based on carboxylic and sulfonic acids with a chain length of four to 18 carbon atoms. Examples are fluorinated alkyl sulfonates, with perfluorooctane sulfonate (PFOS) as the best-known unpleasant representative, and fluorinated carboxylic acids, the most notorious representative of which is perfluorooctanoic acid (PFOA). Fluoroplastics such as Teflon are also counted among PFAS. Tailored PFAS are found in our everyday consumer products from food packaging and cookware to carpets and clothing. They are added to cleaning agents and fire-fighting foams and are widely used industrially, for example in seals, surface coatings and lubricants. When synthesizing such PFAS, hydrogen atoms of organic compounds are replaced with fluorine atoms. This means that the PFAS carbon chain tail is hydrophobic, and the functional head group is hydrophilic. The resulting amphiphilic character explains the use of some PFAS as surfactants. Unlike classic surfactants, these PFAS are lipophobic. This means that they repel water as well as oil and grease. Additional advantages for technical applications include their excellent durability when exposed to heat and aggressive chemicals.

Clear and present danger to our drinking water

PFAS are extremely stable under natural environmental conditions. Since they are not affected by degradation processes, they spread in the environment and PFAS that are present in ionic form can accumulate in water. All PFAS can accumulate in

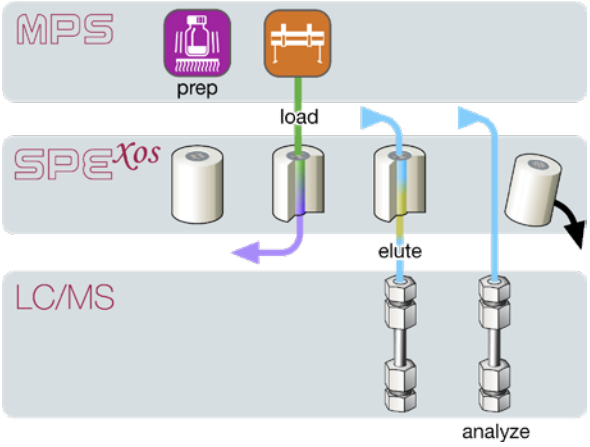


Figure 1: Schematic diagram of the online SPE process with automated cartridge exchange (©GERSTEL)

repositories adjacent to surface water and groundwater, i.e. in and near our most important drinking water reservoirs. The PFAS group of substances includes thousands of substances, 20 of which currently fall under the EU Directive 2020/2184 on the quality of water for human consumption. These 20 substances of particular concern are suspected of causing liver damage, cancer, thyroid disease, obesity, and fertility problems.

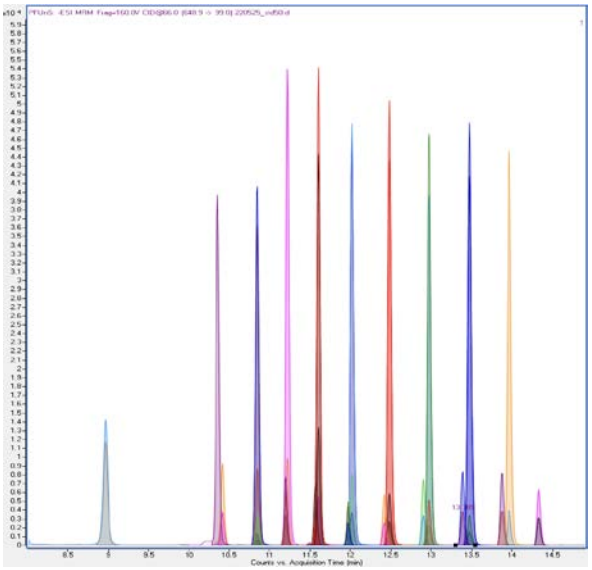


Figure 2: Example chromatogram for a standard solution (50 ng/L) in water with all recorded MRMs. (©GERSTEL)

To minimize the risk of adverse health effects from potentially contaminated drinking water, EU Directive 2020/2184 defines a total limit value of 0.5 micrograms per liter for all PFAS. For the sum of the 20 substances listed in the directive, the maximum limit is 0.1 micrograms per liter. Reliable analysis requires a detection limit of 30 nanograms per liter for the sum of the 20 listed PFAS and 1.5 nanograms per liter for individual compounds.

Analysis based on just one mL of sample

The German standard method for water, wastewater and sludge testing, DIN 38407-42, specifies solid phase extraction (SPE) with subsequent HPLC-MS/MS determination as the method of choice. How efficiently the SPE, and thus the analysis, proceeds depends in no small measure on the solid phase extraction technology used.
Compared to the conventional SPE, which is described in DIN 38407-42, online SPE with GERSTEL SPE^{xos} is based on smaller cartridges. The SPE

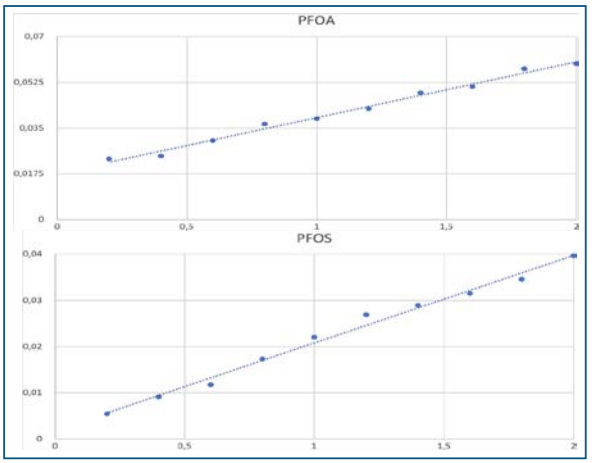


Figure 3: Example calibration curves in the range 0.2 – 2.0 ng/L with (PFOA) and without (PFOS) significant blank. (©GERSTEL)

eluate can be transferred directly and quantitatively to the HPLC column without intermediate. This leads to better limits of detection and -quantification even when a much reduced sample volume is used. Indeed, instead of several hundreds of milliliters, only one mL is required, in turn also reducing the amount of solvent used along with the associated cost and occupational hygiene and environmental impacts.

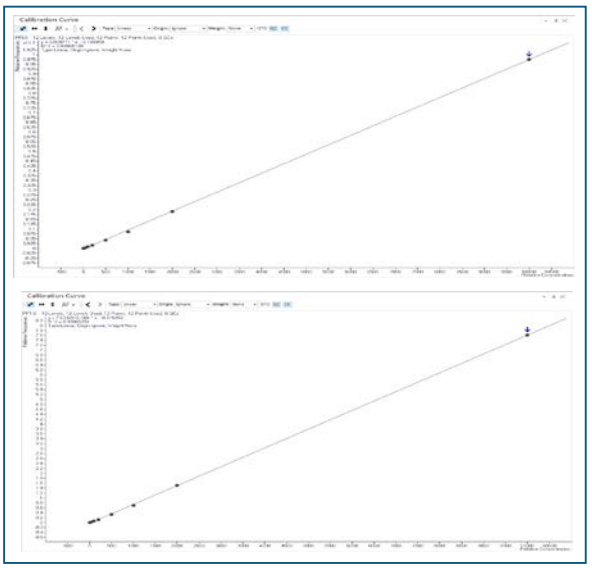


Figure 4: Example calibration curves in the range 1 – 10000 ng/L for the first and the last analyte in the chromatogram.

The use of online SPE in conjunction with a powerful autosampler such as the MultiPurpose Sampler from GERSTEL makes PFAS analysis efficient and convenient. The SPE^{xos} system performs all pre-analysis steps associated with standard SPE sample preparation – from conditioning, loading, rinsing and elution to replacing the cartridges. The MultiPurpose Sampler rinses, and flushes longer chain surface-adsorbed analytes from, the flow path and from the vial walls onto the cartridge. In this way, memory effects can be reduced

to an absolute minimum and excellent recovery of all PFAS compounds is ensured. All this is performed without intervention by laboratory staff. Following analyte elution, SPE^{xos} removes the cartridge from the mobile phase flow path and prepares the system for the next analysis. All the while, HPLC-MS/MS analysis is ongoing. The overlapping sample preparation and analysis runs increase system efficiency and sample throughput without extending the overall analysis time. Parallel processing is controlled by the MAESTRO software and all steps as well as the total analysis time are displayed in the MAESTRO Scheduler for best possible throughput and planning.

Validation round robin test delivers proof of method feasibility

Directly coupling SPE^{xos} with the MultiPurpose Sampler and an HPLC-MS/MS system has proven successful in determining the 20 PFAS listed in EU Directive 2020/2184 as part of a validation trial for the validation of the EN 17892. The new standard EN 17892 describes the LC-MS analysis of PFAS in water using direct injection or solid phase extraction (SPE), which can be performed also as online-SPE. The evaluation of the validation trial was done separately for the different methods, with comparable results for the performance data. GERSTEL participated in the direct injection and online-SPE parts, and all achieved results were consistent with the assigned values.

More detailed information on the online SPE-LC-MS/MS method used by GERSTEL for PFAS water analysis is available in a separate application note (AppNote 237). In it, the applicability of the method and the accuracy of the determination are demonstrated by spiking different water types and performing replicate analyses. According to DIN 32645 the limits of quantification were determined from calibration curves in the range 0.2 to 2 ng/L and were below 1 ng/L for all compounds.

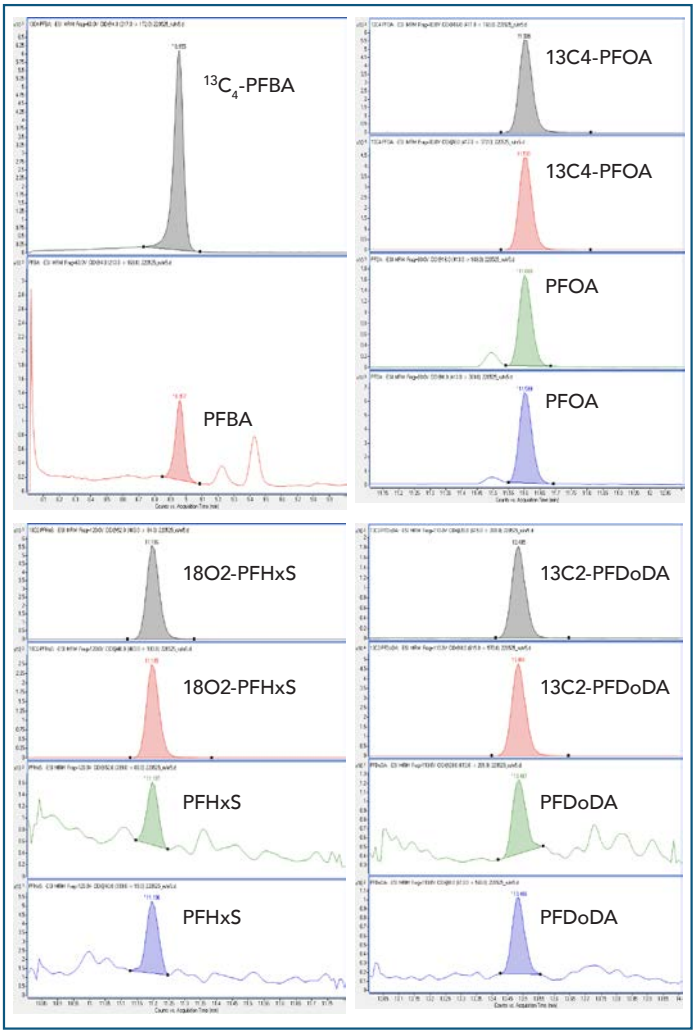
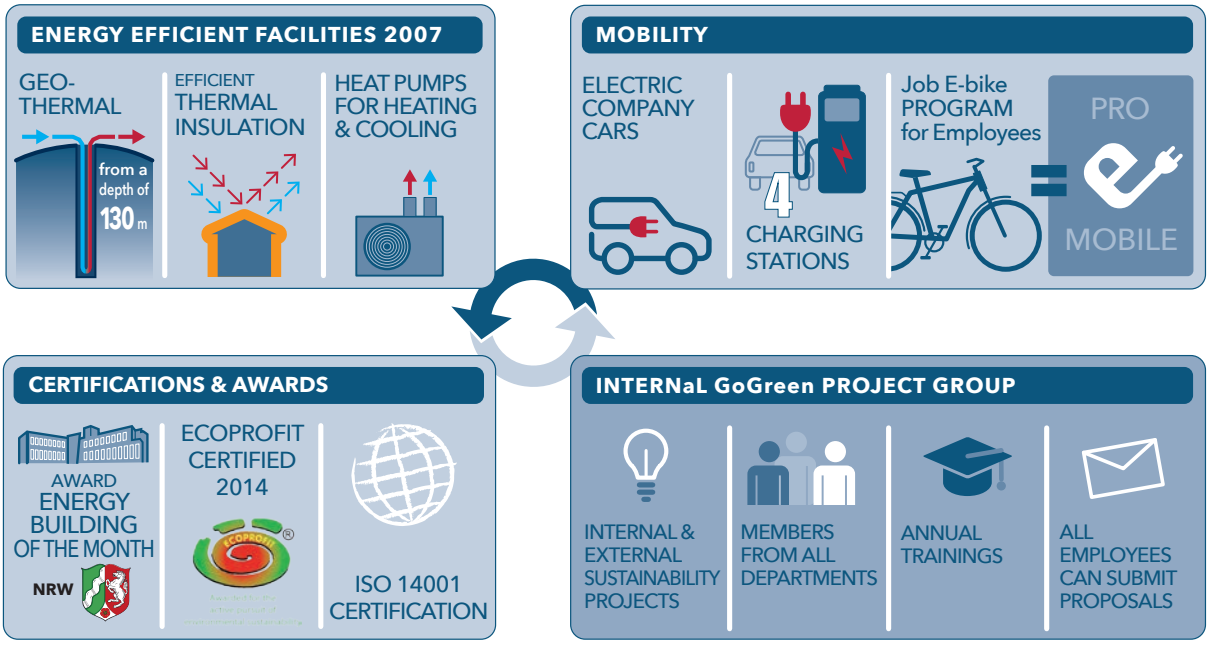


Figure 5: Example chromatograms for selected analytes and internal standards in river water, PFBA and PFOA above, PFHxS and PFDoDA below quantification limit.

More information:

- GERSTEL AppNote 237: Determination of PFAS in Water according to EU 2020/2184 and DIN 38407-42 using online-SPE-LC-MS/MS
- GERSTEL Solutions Worldwide Magazine Nr. 19, 2023, pp. 6-9.
- GERSTEL AppNote 247: Determination of PFAS in Food of Animal Origin using online SPE Cleanup and LC-MS/MS



From Green Beginnings to Green Results: GERSTEL's Journey towards Sustainability

In an era calling for corporations to step up and assume responsibility, GERSTEL emerges as a sustainability trailblazer within the analytical instruments business. The latest result of this long-term commitment is the successful implementation of an Environmental Management System (EMS) certified under DIN EN ISO 14001 standards.

Setting the Stage with ISO 14001

GERSTEL's journey towards environmental sustainability was recently continued and extended with a thorough examination of our operational processes. An internal Go Green team was established with members from departments across the entire operation to identify environmental aspects with potential for improvement across the entire organization. The team's diverse expertise enabled a comprehensive assessment of environmental impact across the organization, from the smallest things like managing greens on the company premises to facilities, logistics, materials and chemicals usage, ultimately achieving ISO 14001 certification. Every facet of GERSTEL's operations was scrutinized to achieve a holistic approach to sustainability.

Ambitious Goals, Tangible Results

With sustainability as a guiding principle, GERSTEL has set ambitious environmental goals, each contributing to a more eco-friendly future. From reducing paper consumption and electronic waste to lowering electricity consumption, the company's commitment is not just about meeting regulatory standards but exceeding them.

GERSTEL's membership in the exclusive club of ISO 14001-certified companies underscores its dedication to environmental responsibility.

GERSTEL
MAKING LABS WORK

The Work Environment

GERSTEL's commitment extends beyond environmental goals. The company actively engages in social initiatives, apprenticeship programs, and continuous employee development. A testament to its dedication to employee well-being, GERSTEL fosters a dynamic work environment with flexible hours, team events, and health initiatives.

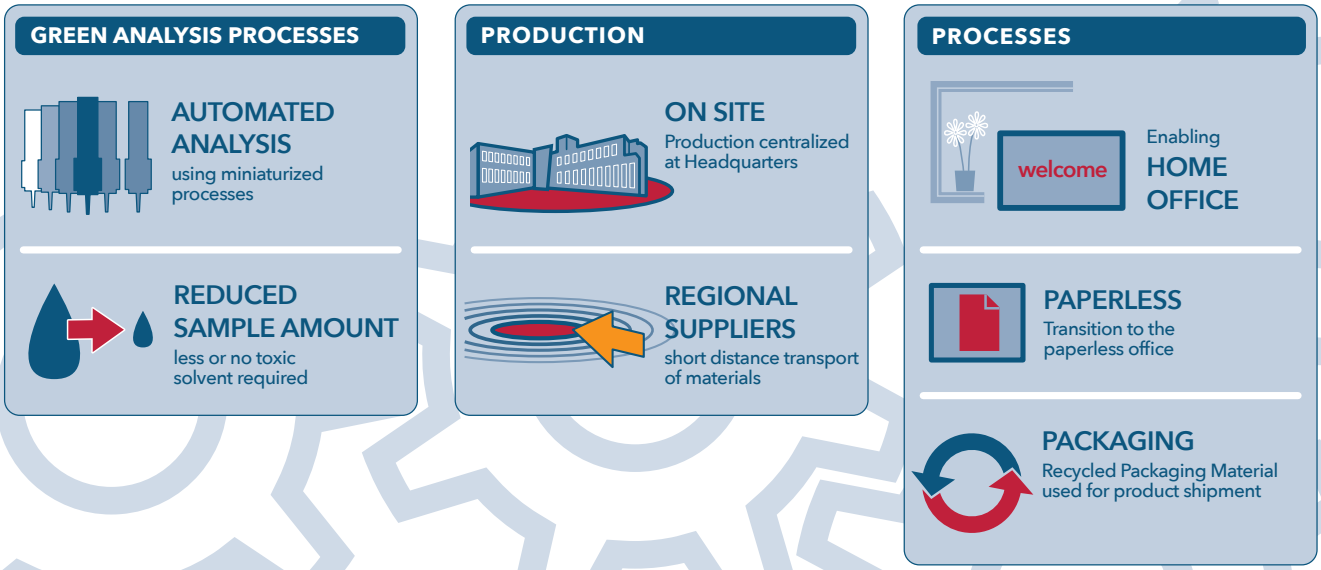
Innovative Practices and Building the Future

The GERSTEL commitment to sustainability is evident not only in its operations but also in its headquarters. The building, constructed in 2007/2008 with state of the art thermal insulation, incorporates sustainable technologies such as geothermal heating and energy-efficient lighting. Current plans include adding a rooftop photovoltaic system. Our commitment to sustainability and environmentally responsible practices run through the entire organization, from product development to building sustainability.

GERSTEL customers benefit from automated miniaturized sample preparation systems that significantly reduce or even eliminate the use of potentially toxic solvents in the laboratory, while delivering outstanding performance, improving the laboratory work environment, and reducing overall cost.

GERSTEL is proud to set an example for the industry, proving that sustainability, environmental responsibility and business success can go hand in hand. By embracing a holistic approach to sustainability, the company positions itself for a sustainable future.

SUSTAINABILITY



Superfood

In search of the fountain of youth: Compounds that keep us young and let us live healthier lifes

By Guido Deussing

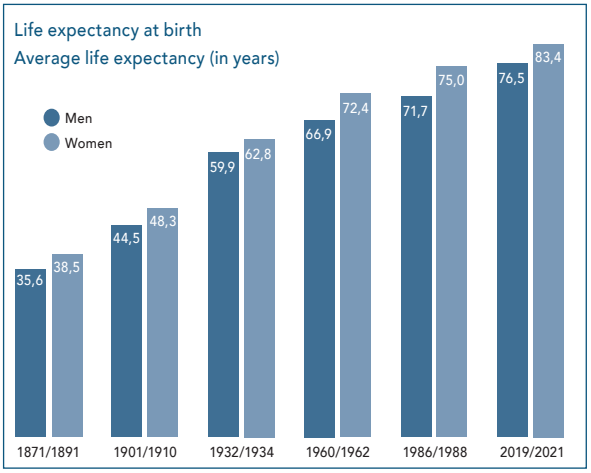
The biogenic polyamine spermidine has received a lot of attention since it was scientifically shown to slow down the aging process when taken in adequate quantities. Determining the spermidine levels in foodstuffs requires the right analysis strategy given that many have a highly complex matrix. Scientists at the Westphalian University in Recklinghausen, Germany have developed an efficient, fully automated method based on HPLC-TOF-MS to determine spermidine in the "superfood" Apilarnil.

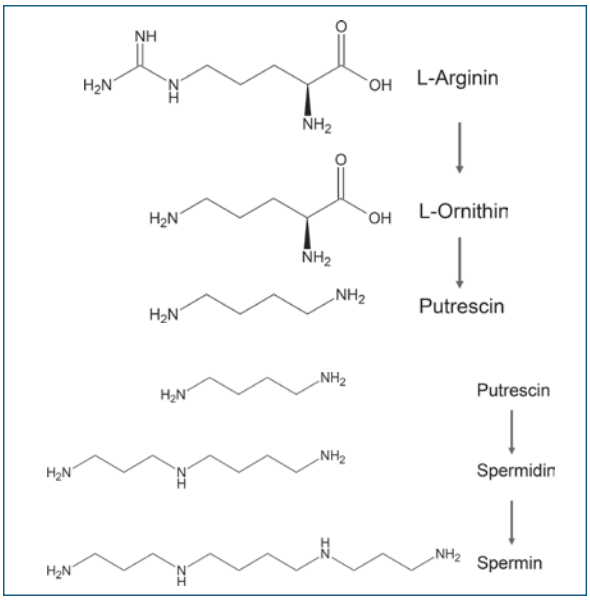
Wish and Reality

According to the Federal Statistical Office, life expectancy in Germany has almost doubled in the past 100 years: Girls born between 2020 and 2022 will live to an average of 83.2 years. On average, boys die five years earlier at 78.3 years old. But that doesn't for a minute stop us from wanting to live even longer and healthier lives. Life can be understood as a natural process, which after a point is characterized by progressive impairment of cell functions. Aging is part of life, but can it be slowed down?

Eat, Drink and be Merry

Regarding what determines our lifespan and quality of life, what we eat seems to be of great importance. There is widespread belief among nutritional experts that the Mediterranean diet, for example, has a positive effect on our health and can prolong life [1]. The diet includes large helpings of fruits and vegetables, as well as legumes, wholegrain products, and olive oil; add fish, milk and wine in moderation and meat in small quantities. Anti-agers and longevity seekers, among many others, want to know what makes this diet so valuable. It has been scientifically proven that the intake of certain foods has a positive effect on a person's lifespan.





Breaking Bad Habits

According to a Norwegian study, it is worth breaking bad eating habits even in old age, for example, avoiding fast food, eating less meat, and instead eating more legumes and whole grain products. After finishing their study [2], Fadnes et al. concluded that a 20-year-old can gain more than ten years of life with the right diet, and an 80-year-old more than three years of added life. At number one on the list of valuable foods are legumes such as lentils, beans, and peas. According to Fadnes et al., whole grain products and nuts are just as healthy as legumes. Fruit, vegetables, and fish also have a positive influence on a person's potential lifespan. Red meat and processed products such as sausage and ham have a negative impact, while eggs, poultry, processed cereals, and sugary drinks don't have much impact. Foods with antioxidant and anti-inflammatory properties counteract cell damage and age-related ailments such as cardiovascular disease, cancer, diabetes, and neurodegenerative diseases, such as Alzheimer's. Given that not everyone is able to eat the optimal amount of healthy food day in and day out, adding suitable dietary supplements is sensible. The European Food Safety Authority (EFSA) for one considers it helpful to compensate for nutritional deficiencies by maintaining an appropriate intake of relevant nutrients [3].

Cleaning and Recycling

One age-related change in the human body is that cellular cleansing processes deteriorate. The so-called autophagy is a type of recycling system that breaks down and removes unnecessary or damaged cell components, explains Evgeni Ponimaskin. This molecular clean-up mechanism keeps cells fit and protects against many diseases. However, as we age, the autophagy process stalls, but can apparently be reactivated with the compound spermidine, as the researcher from the Institute of Neurophysiology at the Hannover Medical University (MHH) states [4].

Picture caption (left hand side): Spermidine is an endogenous, natural substance, a polyamine first discovered in human semen, which gave the substance its name. We now know that spermidine is found in all cells in the body and that certain intestinal bacteria produce it as shown here. However, the majority must be absorbed through food. The foodstuffs that are known to contain the largest amounts of spermidine are wheat germ, cheese, especially Cheddar, soy products and legumes, and most of all in Apilarnil.

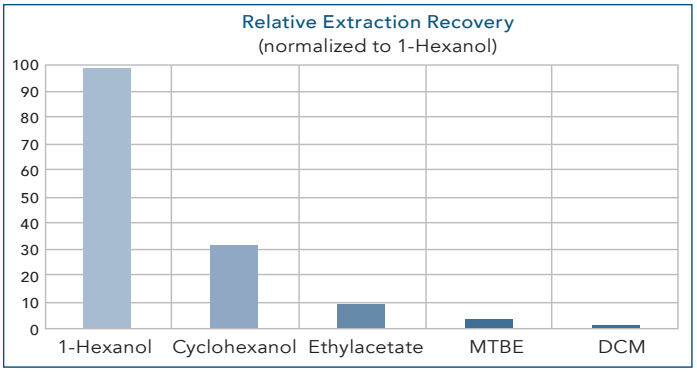


Figure X: When extracting spermidine from Apilarnil, 1-hexanol achieved the highest relative yield.

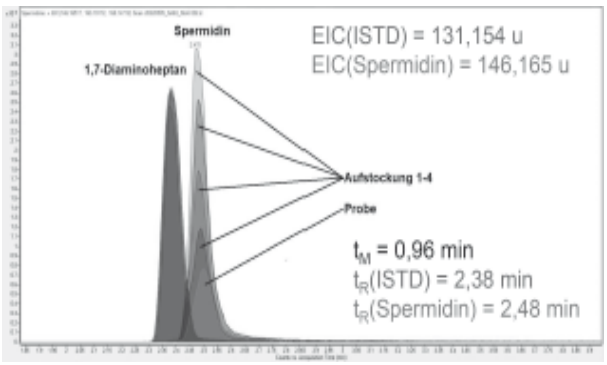
Heart and Mind

The anti-aging effect of spermidine has been examined in more detail by Ponimaskin and his research group in cooperation with the University of Graz based on animal experiments [3,4]. The researchers administered spermidine through drinking water to aged mice for six months and compared the results after the feeding period with those of untreated animals of the same age. As Ponimaskin and his team report, clear anti-aging effects were observed in the treated animals. The spermidine intake ensured that the animals developed less kidney and liver damage and improved the performance-enhancing glucose supply in the brain. Age-related hair loss was also significantly lower than in the control group. The animals supplied with spermidine hardly showed any bald spots on their backs as normally seen in older mice.

The heart-protecting effect of spermidine was particularly interesting to the scientists. In their studies, they report finding that the cardioprotective effect

is associated with reduced telomere shortening in heart muscle tissue. Telomeres protect the ends of chromosomes, carriers of genetic information stored in our body cells, from degeneration. Each time a cell divides, the telomere ends are shortened slightly. In cells that are no longer dividing - such as heart muscle cells - the telomeres are further shortened until a critical length is reached and so-called programmed cell death occurs. To their delight, the researchers found that telomeres in the spermidine-supplemented mice were similar in length to those in young animals. Since the aging processes in the cells of mice are similar to those in human cells, taking spermidine, for example as a dietary supplement, could also protect against age-related problems in humans, while strengthening cognitive processes and memory. To check whether the results from the animal model can be transferred to humans, researchers from Austria and Germany [6,7] used data from the Bruneck study. A group of 829 participants with normal cognitive performance in 1995 was selected. Those of the subjects who had developed cognitive impairments over the following five years of observation were identified using the neuropsychological test battery CERAD (Consortium to Establish a Registry for Alzheimer's Disease). The domains of memory, executive functions (planning) and language skills in the brain were examined. Spermidine intake from food was then determined. The results show that study participants who ingested more food with high spermidine levels, such as wholegrain products and legumes, in 1995 showed significantly less cognitive loss over the following five years.

Nutritional supplements containing alpha-ketoglutarate, hyaluronic acid, NAD+ boosters, vitamin D3 and vitamins B7, B9 and B12 are advertised as "longevity products". As part of his rejuvenation treatment, the billionaire Bryan Johnson takes 13.5 mg of spermidine in his "The Green Giant" smoothie every morning, apparently with some success in reversing the aging process. The "Blueprint" rejuvenation project can be followed on his Webpage [5]. As recent studies suggest [4], spermidine does appear to influence the aging process.



Experiments and Evidence

Various studies have produced reliable evidence of positive effects of spermidine on human health. Examples are: Improved autophagy, reduced risk of cancer, improved cardiac health, effective protection against Alzheimer's and Parkinson's, reduced risk of developing type 2 diabetes, as well as a reduction in high blood pressure, as Prof. Dr. Ingo Tausendfreund from the Westphalian University in Rechlinghausen, Germany explains. The Bruneck study by research teams from Graz and Innsbruck with 829 test subjects even showed that people whose diet was rich in spermidine had a significantly lower risk of dying over the 20-year observation period.

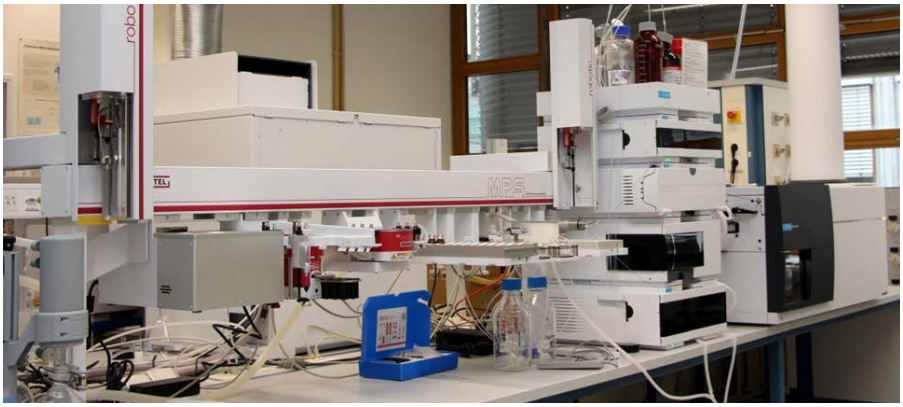
Valuable and Content Rich

Foodstuffs that are rich in spermidine have been categorized as "superfoods", to be used for life-prolonging and rejuvenation treatment plans. The following foodstuffs are valuable sources of spermidine (Spermidine content listed per 100 g): avocado (1.0 mg), potato (1.2-1.7 mg), hazelnuts (2.1 mg), mango (3,0 mg), cauliflower and broccoli (2.6-3.7 mg), pear (5.2 mg), peas (6.5 mg), shiitake mushrooms (8.9 mg), aged cheddar (19.9 mg) and wheat germ (24.0 mg). Those more interested in business aspects can take note that a market analysis for "superfoods" has projected growth from 165 billion US dollars in 2023 to 269 billion US dollars in 2028 [8].

Speaking of superfoods: Beekeepers (apiarists) in Germany offer ostensibly performance enhancing bee preparations under the name Apilarnil an extract of freeze-dried and powdered drone larvae, which contains spermidine, beta-carotene, choline, vitamins A, E, B1, B2, and B6, as well as numerous minerals (calcium, phosphorus, sodium, potassium, and magnesium) along with trace elements. The discovery that drone larvae extract can be a valuable nutritional supplement came from a chance observation: Ducklings fed drone honeycombs grew faster than a reference group.

Bee Larvae Prepared

A company specializing in the marketing of apiary products approached to determine the spermidine content in freeze dried drone larvae, a challenging task due to the complex biological matrix. A completely automated approach was required to process large numbers of samples. LC-MS/MS was identified as the method of choice; diode array detection (DAD) would require considerable added sample prepara-



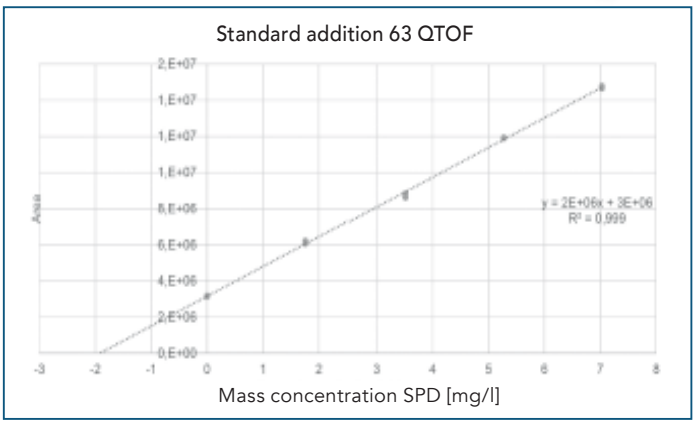
tion effort and derivatization steps with added uncertainty. The planned analysis steps were: 1. Selective extraction of the biogenic amine spermidine from the matrix; 2. Chromatographic separation; 3. Determination with a quadrupole time-of-flight (QTOF)-MS.

In initial work, 1-hexanol was found to produce the highest relative extraction yield as shown in the Figure on page 21. 1,7-diaminoheptane was added as internal standard in a standard addition process. Using the GERSTEL MultiPurpose Sampler (MPS) robotic, the researchers performed preliminary tests, optimizing the process regarding the sample amount, internal standards used, calibration strategy, and various method parameters (volumes, mixing times, centrifugation, wash cycles, needle penetration depths, etc. (For details on the method, please contact info@gerstel.de).

The MPS robotic, equipped with quickMIX and centrifuge enabled complete automation of all steps in the workflow from sample preparation to sample introduction. The analysis was performed using an Agilent 1260 UHPLC with a Restek Force Fluorophenyl column (3.0 µm, 150 x 2.1 mm) using a solvent gradient. Mass-selective detection was performed using an Agilent QTOF 6546 (Dual AJS ESI source) in positive mode (TOF-only). The analysis can also be performed using an LC-MS/MS system (precursor ion m/z 146.00, daughter ion m/z 72.00).

Results and Outlook

A series of apilarnil samples were analyzed with spermidine levels ranging from 310 to 459 mg/kg, averaging 386 mg/kg. The relative standard deviation of the determination was 1.5 percent with linearity (R^2) greater than or equal to 0.999. The analysis method was successfully developed and automated based on the MPS robotic-LC-QTOF system. The following are amounts of spermidine per 100 g: Peas: 6.5 mg; aged cheddar: 19.9 mg; wheat germ: 24.0 mg. Apilarnil weighed in at a whopping 38.6 mg per 100 g. Whether it can extend our life expectancy – or keep us (feeling) young remains to be seen.



Standard Addition calibration curve for spermidine in Apilarnil demonstrating excellent linearity

The automated method can be used to determine spermidine levels in other foodstuffs if the buzz surrounding spermidine continues. The hope is that finding other sources of this promising nutrient will ultimately lead to more and extended well-being for the wider population.

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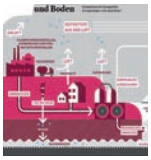

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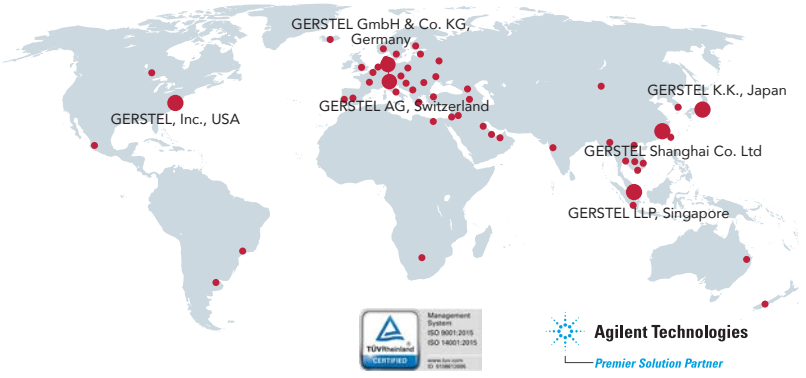

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